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A Comparative Study of the Activity of Lysosomal and Main Metabolic Pathway Enzymes in Tissue Biopsies and Cultured Fibroblasts from *Dupuytren*'s Disease and Palmar Fascia

On the pathobiochemistry of connective tissue proliferation, I.

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Respectfully dedicated to Prof. Dr. Fritz Hartmann, Hannover, on the occasion of his 60th birthday

Summary: Activities of ten main metabolic pathway enzymes and seven lysosomal enzymes were determined in specimens from human normal palmar fascia and *Dupuytren*'s contracture. The activities of the enzymes tested are 2–3 times higher in fresh specimens of *Dupuytren*'s contracture. There are no differences in the activity distribution patterns of both these specimens, or in the absolute activities calculated in relation of the glyceraldehyde-3-phosphate dehydrogenase activities. With the exception of adenylate kinase and pyruvate kinase, the activities of main metabolic pathway enzymes based on the DNA content showed significantly lower activities in *Dupuytren*'s contracture tissue than in palmar fascia. Lysosomal enzymes exhibit no significant differences of activity in the respective specimens. However, the lysosomal enzyme activities of cultured fibroblasts are lower than the corresponding activities from tissue specimens. The enzyme activities per DNA content in cultured fibroblasts are 10–50 times higher than in tissue specimens. The enzyme activities in cultured fibroblasts decrease with age or density of the cells in culture. The increased metabolic activity of the diseased tissues in *Dupuytren*'s contracture is due to the higher cell content of the afflicted portions of the tissue, but individual enzymes show no qualitative changes in activity and there are no increases of enzyme activity per cell (DNA).

Eine vergleichende Untersuchung über die Aktivitäten lysosomaler und Hauptketten-Enzyme in Biopsiematerial und Fibroblasten in der Gewebekultur von Palmarfascie und Dupuytren'schem Kontrakturgewebe

Zur Pathobiochemie der Bindegewebsproliferation, 1. Mitteilung

Zusammenfassung: Die Aktivitäten von 10 Hauptkettenenzymen und sieben lysosomalen Enzymen wurden in Gewebsproben von normalen menschlichen Palmaraponeurosen und von *Dupuytren*'scher Kontraktur bestimmt. Die ermittelten Enzymaktivitäten lagen in den frischen Proben der *Dupuytren*'schen Kontraktur 2–3 mal höher als in Gewebe von Palmarfaszie. Differenzen in der relativen Enzymverteilung zwischen den Proben von *Dupuytren*'scher Kontraktur und Palmarfaszie konnten nicht festgestellt werden. Ebenfalls fanden sich keine signifikanten Unterschiede beim Bezug der Enzymaktivitäten auf die Glycerinaldehydphosphatdehydrogenase als Bezugssystem. Die Aktivitäten von Hauptkettenenzymen im Bezug auf den DNA-Gehalt der Gewebsproben waren im *Dupuytren*'schen Kontrakturgewebe signifikant niedriger als in dem Vergleichsmaterial der Palmaraponeurose mit der Ausnahme von Adenylatkinase und Pyruvatkinase. Lysosomale Enzyme zeigten keine signifikanten Unterschiede in ihrer Aktivität in den entsprechenden Geweben. In Fibroblasten, welche aus den entsprechenden Gewebsproben gezüchtet wurden, waren die Aktivitäten lysosomaler Enzyme vergleichsweise niedriger als in den zugehörigen Gewebsproben. Die Aktivitäten in den Fibroblasten im Bezug auf den DNA-Gehalt fanden sich 10–50 mal höher als in den entsprechenden Gewebsproben. Die Enzymaktivitäten in Fibroblasten nehmen mit zunehmender Dichte und Alter der Kultur ab. Die erhöhte metabolische Aktivität der betroffenen Gewebspartien bei der *Dupuytren*'schen Kontraktur muß auf den erhöhten Zellgehalt der Gewebe zurückgeführt werden und geht nicht mit qualitativen Veränderungen der Aktivitäten einzelner Enzyme oder einer Zunahme der Enzymaktivitäten in den Zellen einher.

Introduction

Malfunction of tissue repair plays a major role when considering pathological states of connective tissues. In addition to those connective tissue diseases which are characterized by lack of defence or repair reactions, other cases are characterized by uncontrolled tissue proliferation leading to severe destruction and malfunction of connective tissue structures. The progressive con-

[1]) This publication contains data from the Doctoral Thesis of *E. Reimers* and *I. Schönborn*.

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tracture of palmar and plantar fascia first described in 1614 by *E. Plater* (1) and investigated in 1832 by *Dupuytren* (2) is morphologically characterized by heavy cell proliferation and destruction as well as rebuilding of fibre proteins (3). In contrast to the large number of publications on the morphological changes of the tissue structure in *Dupuytren* disease, only a few papers have been published on the pathobiochemistry of this disorder. They indicate an increased collagen and glycosaminoglycan content and metabolism of the afflicted tissue portions (4–8).

Data from an earlier study performed in our laboratory on connective tissue demonstrated an increase in the activity of main metabolic pathway enzymes in *Dupuytren* tissue (11). The enzyme activity pattern showed a similar distribution to that found in other connective tissues, with relatively high activities of phosphate transferases. During the course of our investigation, *Hoopes* et al. published data on enzymes of glucose metabolism in *Dupuytren* contracture and the adjacent skin showing elevated activities in both these tissues (12). The goal of the present study was the confirmation of our earlier results on a larger group of patients with *Dupuytren* contracture. Furthermore a comparative study of enzyme activities in cultured fibroblasts grown from normal palmar aponeurosis and *Dupuytren* contracture should reveal basic data for the interpretation of in-vitro experiments on the pathobiochemistry of connective tissue proliferation.

The enzymes[2]) chosen for activity determination were lactate dehydrogenase representing *glycolysis*, glyceraldehyde-3-phosphate dehydrogenase for the *Embden-Meyerhof* pathway, adenylate kinase, pyruvate kinase and 3-phosphoglycerate kinase as *phosphate transfer* enzymes, malate dehydrogenase and isocitrate dehydrogenase for the *Krebs cycle*, glucose-6-phosphate dehydrogenase for the *hexose monophosphate shunt*, glutamate-oxalacetate transaminase, glutamate-pyruvate transaminase and glutamate dehydrogenase for *amino acid metabolism*, collagen peptidase and cathepsin B 1 representing *collagen breakdown* and β-glucuronidase, α-N-acetyl-glucosaminidase, α-*L*-fucosidase, arylsulfatase for *carbohydrate decomposition*, and acid phosphatase.

Material and Methods

Tissue specimens

Tissue specimens were taken during surgery from patients aged 40–70 years suffering from *Dupuytren*'s contracture of the hand. Normal aponeurosis specimens are obtained by autopsy within 24 h post mortem. The specimens were freed from adjacent tissue and blood, cut into small pieces and homogenized with an ultra-turrax-homogenizer (Janke and Kunkel, Stauffen, Germany) at 4 °C in 0.15 mol/l KCl, 1 ml/l Trition X 100 ten times for 15 minutes, followed by 30 minutes intermission to avoid an increase in temperature. The homogenate was extracted for 60 minutes by stirring at + 4 °C followed by a second homogenisation as above. The extract was cleared by centrifugation at 100000 *g* for 30 minutes (Superspeed 50, MSE Ltd., London SW 1, England). The pellet was extracted by acetone for dry weight estimation and DNA assay.

The biopsy specimens were macroscopically examined and the nodular portions selected for the extraction and enzyme activity determinations (in the following referred to as *Dupuytren's contracture*).

One part of these specimens underwent microscopic examination and could be characterized as cell-rich proliferating tissue (*proliferating stage*). From suitable specimens thin unsuspected portions of biopsies were collected. They showed microscopically no or only slight proliferation (*thin portion of Dupuytren's contracture*).

The reference group (*palmar fascia*) exhibited a normal microscopical tissue structure.

Enzyme activity determinations

Enzyme activity determination was performed in triethanolamine buffer 50 mmol/l and ethylenediamine tetraacetate (disodium salt) 3 mmol/l, pH 7.5 at 25 °C and measured at the appropriate wavelength in the Photometer Eppendorf (Eppendorf Gerätebau GmbH, Hamburg, Germany) or the Beckman 25 Spectrophotometer (Beckman Instruments GmbH, Munich, Germany) if not otherwise stated.

The substrate concentrations in the tests were:

Lactate dehydrogenase

NADH 0.1 mmol/l, $MgCl_2$ 5 mmol/l, pyruvate 1.25 mmol/l

Glyceraldehyde-3-phosphate dehydrogenase

NADH 0.1 mmol/l, glutathione (GSH) 2.5 mmol/l, $MgSO_4$ 10 mmol/l, 3-phosphoglycerate 3 mmol/l, phosphoglycerate kinase 4000 U/l, ATP 5 mmol/l

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[2]) Enzymes

Adenylate kinase (ATP: AMP phosphotransferase, EC 2.7.4.3)

Acid phosphatase (Orthophosphoric-monoesterphosphohydrolase, EC 3.1.3.2)

Arylsulfatase (EC 3.1.6.1)

Cathepsin B1 (EC 3.4.22.2)

Collagen peptidase (EC 3.4.4.-)

α-*L*-Fucosidase (EC 3.2.1.51)

Glyceraldehyde-3-phosphate dehydrogenase (*D*-Glyceraldehyde-3-phosphate: NAD oxidoreductase phosphorylating, EC 1.2.1.12)

α-N-Acetyl-glucosaminidase

Glutamate dehydrogenase (*L*-Glutamate: NAD (P)oxidoreductase (deaminating), EC 1.4.1.3)

β-Glucuronidase (β-*D*-Glucuronide-glucuronohydrolase, EC 3.2.1.31)

Glutamate-oxalacetate transaminase (*L*-Aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1)

Glutamate-pyruvate transaminase (*L*-Alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2)

Glucose-6-phosphate dehydrogenase (*D*-Glucose-6-phosphate: NADP oxidoreductase EC 1.1.1.49)

Isocitrate dehydrogenase (threo-*D*-Isocitrate: NADP oxidoreductase (decarboxylating) EC 1.1.1.42)

Lactate dehydrogenase (*L*-Lactate: NAD oxidoreductase, EC 1.1.1.27)

Malate dehydrogenase (*L*-Malate: NAD oxidoreductase, EC 1.1.1.37)

Pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40)

3-Phosphoglycerate kinase (ATP: 3-phospho-*D*-glycerate 1-phosphotransferase EC 2.7.2.3)

Pyruvate kinase

NADH 0.25 mmol/l, phosphoenolpyruvate 2.5 mmol/l, $MgSO_4$ 10 mmol/l, KCl 100 mmol/l, lactate dehydrogenase 7200 U/l, ADP 1.25 mmol/l

Adenylate kinase

NADH 0.1 mmol/l, $MgSO_4$ 50 mmol/l, KCl 50 mmol/l, phosphoenolpyruvate 1.25 mmol/l, lactate dehydrogenase 18000 U/l, pyruvate kinase 6000 U/l, ATP 1 mmol/l, AMP 1 mmol/l

Malate dehydrogenase

NADH 0.2 mmol/l, oxalacetate 0.2 mmol/l

Glucose-6-phosphate dehydrogenase

NADH 0.5 mmol/l, glucose-6-phosphate 2.4 mmol/l

Isocitrate dehydrogenase

NADP 0.3 mmol/l, $MnCl_2$ 5 mmol/l, isocitrate 1.9 mmol/l

Glutamate-oxalacetate transaminase

NADH 0.25 mmol/l, 2-oxoglutarate 9 mmol/l, KCl 30 mmol/l, malate dehydrogenase 22000 U/l, *L*-aspartate 0.1 mol/l, phosphate buffer 50 mmol/l, pH 7.5

Glutamate-pyruvate transaminase

NADH 0.3 mmol/l, 2-oxoglutarate 11.25 mmol/l, lactate dehydrogenase 9000 U/l, *L*-alanine 0.3 mol/l

Glutamate dehydrogenase

ADP 1.0 mmol/l, NADH 0.15 mmol/l, ammonium sulphate 80 mmol/l, 2-oxoglutarate 8 mmol/l

3-Phosphoglycerate kinase

NADH 0.1 mmol/l, EDTA 2.6 mmol/l, glycerate-3-phosphate 6.0 mmol/l, $MgSO_4$ 7.5 mmol/l, glutathione (GSH) 2.5 mmol/l, glyceraldehyde-3-phosphate dehydrogenase 7000 U/l, ATP 2.5 mmol/l

The lysosomal enzyme activity determinations were carried out as follows:

Arylsulfatase

Sodium acetate buffer 72 mmol/l, pH 6.2; 4-nitrophenyl-hydrogensulphate potassium salt 10 mmol/l, incubation for 60 min at 37 °C. Reaction stopped by addition of NaOH final concentration 1.33 mmol/l. Photometer Eppendorf 405 nm ((13), modified)

β-Glucuronidase

Sodium acetate buffer 96 mmol/l, nitrophenyl-β-*D*-glucuronide 10 mmol/l, pH 4.8, incubation for 60 min at 37 °C. Reaction stopped by addition of glycine carbonate final concentration 133 mmol/l, pH 10. Photometer Eppendorf 405 nm

α-N-Acetyl-glucosaminidase

Sodium citrate buffer 39 mmol/l, pH 4.2, *p*-nitrophenyl-2-acetamido-2-deoxy-α-*D*-glucopyranoside 7.98 mmol/l, incubation for 60 min at 37 °C. Reaction stopped by addition of glycine NaOH buffer, final concentration 320 mmol/l, pH 10.4. Photometer Eppendorf 405 nm (15)

α-L-Fucosidase

Citrate phosphate buffer 53 mmol/l, pH 6.1, *p*-nitrophenyl-*D*-fucoside 0.5 mmol/l, incubation for 60 min at 37 °C. Reaction stopped by addition of hydrogen carbonate buffer final concentration 100 mmol/l, pH 10.6. Photometer Eppendorf 405 nm (16).

Collagen peptidase

Tris-HCl buffer 120 mmol/l, pH 7.2, 4-phenylazobenzyl-oxycarbonyl-*L*-prolyl-*L*-leucyl-glycyl-*L*-prolyl-*D*-arginine-dihydrate 0.41 μmol/l, incubation 60 min at 37 °C. Reaction stopped by addition of citric acid, final concentration 30 mmol/l, extraction of the reaction product with benzene. Photometer Eppendorf 334 nm (17).

Cathepsin B 1

Hydrogencarbonate/carbonate buffer 190 mmol/l, pH 6.0, Ethylenediaminetetraacetate disodium salt 0.95 mmol/l, cysteine hydrochloride 1.9 mmol/l, α-benzoyl-*D*, *L*-arginine-2-naphthylamide 2.19 mmol/l in dimethylsulfoxide, incubation for 10 min at 25 °C. Photometer Eppendorf 340 nm (18, 19)

Acid phosphatase

Citrate buffer 40 mmol/l, pH 4.8; Na-*p*-nitrophenylphosphate 4.6 mmol/l, 37 °C. Photometer Eppendorf 405 nm (20).

Enzyme activities are expressed in U (μmol substrate turnover per minute under the given conditions).

Protein assay: was performed according to *Weichselbaum* (21) in extracts of biopsy specimens; and by the method of *Lowry* et al. (22) for autopsy and fibroblast extracts.

Blood contamination: was calculated by haemoglobin determination according to *Crosby & Furth* (23).

DNA determination: modified according to *Burton* (24). Following enzymatic proteolysis by papain (25), precipitation of DNA by $HClO_4$ (final concentration 1 mol/l) and centrifugation at 2400 *g* 15 minutes. Hydrolysis of the pellet in 0.5 mol/l HCl at 70 °C for 20 minutes, photometry with diphenylamine reagent at 600 nm Beckman Spectrophotometer 25.

Hydroxyproline assay: according to *Stegemann* (26).

Cell culture conditions: minimum essential medium with *Earl*'s salt and 10% fetal calf serum at 37 °C; 95% air/5% CO_2 (25).

Cell extract: cells are freed from culture flasks by pronase, washed with isotonic sodium chloride and resuspended in 0.15 mol/l KCl containing 0.1 ml/l Triton X 100 (27) and 2.5 mmol/l ethylenediaminetetraacetate disodium salt. An aliquot of the suspension was taken for cell counting in the TOA cell counter (Colora Meßtechnik Lorch, Germany). The remaining suspension was treated for 10 minutes by ultrasonic irradiation, centrifuged for 20 minutes at 100000 *g*. The supernatant was used for enzyme activity determination and protein assay.

Results

Enzyme activity determinations in strong tissues such as palmar fascia depend as much on the method used to disrupt the tissue structure and to extract the homogenates as on the test conditions chosen for the activity assays. The methods applied in this study include the addition of triton X 100 to the extraction medium which resulted in extraction rates five times higher for lysosomal enzymes, while no effect in the yield of main metabolic enzymes could be observed. Triton X 100 did not interfere with the activity estimation of these enzymes. Repeated breakdown and extraction of the tissue specimens leads to a gain of less than 10% of the enzyme activities present in the first extract; only the first extract was taken for enzyme determinations in this study. The extraction of cultured fibroblasts is practically complete; after extraction, cell residues show no measurable activity of main metabolic pathway enzymes or lysosomal enzymes.

For interpretation of the data on the enzyme activities in the different specimens, wet weight, dry weight, DNA

and hydroxyproline content and the amount of extractable protein were determined (tab. 1A). Dry weight and hydroxyproline content do not differ between *Dupuytren*'s contracture and palmar fascia. The extracted protein values of *Dupuytren*'s contractures are found to be twice as high as those of palmar fascia and the DNA content about four times higher in *Dupuytren*'s contracture than in palmar fascia. The respective data for the thin portion of *Dupuytren* tissue are between those for *Dupuytren*'s contracture and palmar fascia values. The statistical significance of these findings is listed in table 1B. Enzyme activity represented by the activity of glyceraldehyde-3-phosphate dehydrogenase follows the DNA content of the specimens (fig. 1) in the palmar fascia and *Dupuytren*'s contracture. The macroscopically classified nodular portions (*Dupuytren*'s contracture, fig. 1) and the tissue specimens which were histologically classified as proliferating state of *Dupuytren*'s contracture showed no significant differences in dry weight, extractable protein, hydroxyproline or DNA content. The results of the enzyme activity determinations are summarized in figure 2 and tables 2A and 2B. The activity distribution patterns are characterized by high activities of lactate dehydrogenase, malate dehydrogenase and phosphotransferases while the activities of the transaminases, glutamate dehydrogenase and glucose-6-

Tab. 1. Deoxyribonucleic acid content, dry weight, extracted protein and hydroxyproline content of palmar fascia and *Dupuytren*'s contracture (subgroups). A: content per kg wet weight. B: significance level of differences between the specimen groups.

A	Palmar fascia		Nodular portion *Dupuytren*'s contracture		Thin portion *Dupuytren*'s contracture		Proliferating stage	
	n	$\bar{x} \pm s_{\bar{x}}$	n	$\bar{x} \pm s_{\bar{x}}$	n	$\bar{x} \pm s_{\bar{x}}$	n	$\bar{x} \pm s_{\bar{x}}$
DNA (g/kg wet weight)	13	0.2 ± 0.02	28	0.62 ± 0.05	12	0.39 ± 0.05	11	0.54 ± 0.08
Dry weight (kg/kg wet weight)	15	0.21 ± 0.03	30	0.19 ± 0.009	11	0.17 ± 0.01	11	0.21 ± 0.03
Extracted protein (g/kg wet weight)	17	1.34 ± 0.1	27	2.15 ± 0.16	13	1.97 ± 0.2	12	2.0 ± 0.12
Hydroxyproline (g/kg wet weight)	13	24.7 ± 2.2	27	23.9 ± 1.2	10	21.3 ± 1.8	10	22.5 ± 2

B	p		p		p		p	
Palmar fascia	DNA		0.000		0.001		0.01	
	Dry weight		0.67		0.19		0.34	
Nodular portion *Dupuytren*'s contracture	0.000		Protein	DNA	0.003		0.53	
	0.63		Hydroxyproline	Dry weight	0.28		0.50	
Thin portion *Dupuytren*'s contracture	0.002		0.49		Protein	DNA	0.11	
	0.67		0.24		Hydroxyproline	Dry weight	0.41	
Proliferating	0.000		0.41		0.96		Protein	
	0.81		0.85		0.49		Hydroxyproline	

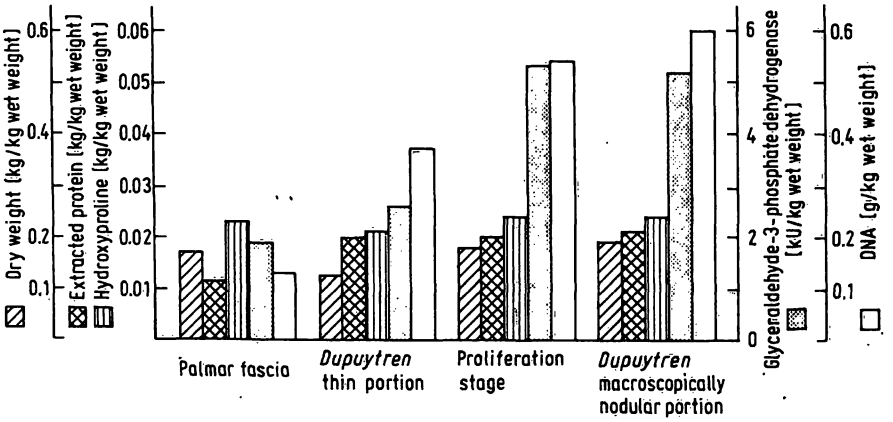

Fig. 1. Comparison of dry weight, extracted protein, hydroxyproline, deoxyribonucleic acid contents and glyceraldehyde-3-phosphate dehydrogenase activity in fresh specimens from palmar fascia and *Dupuytren*'s disease.

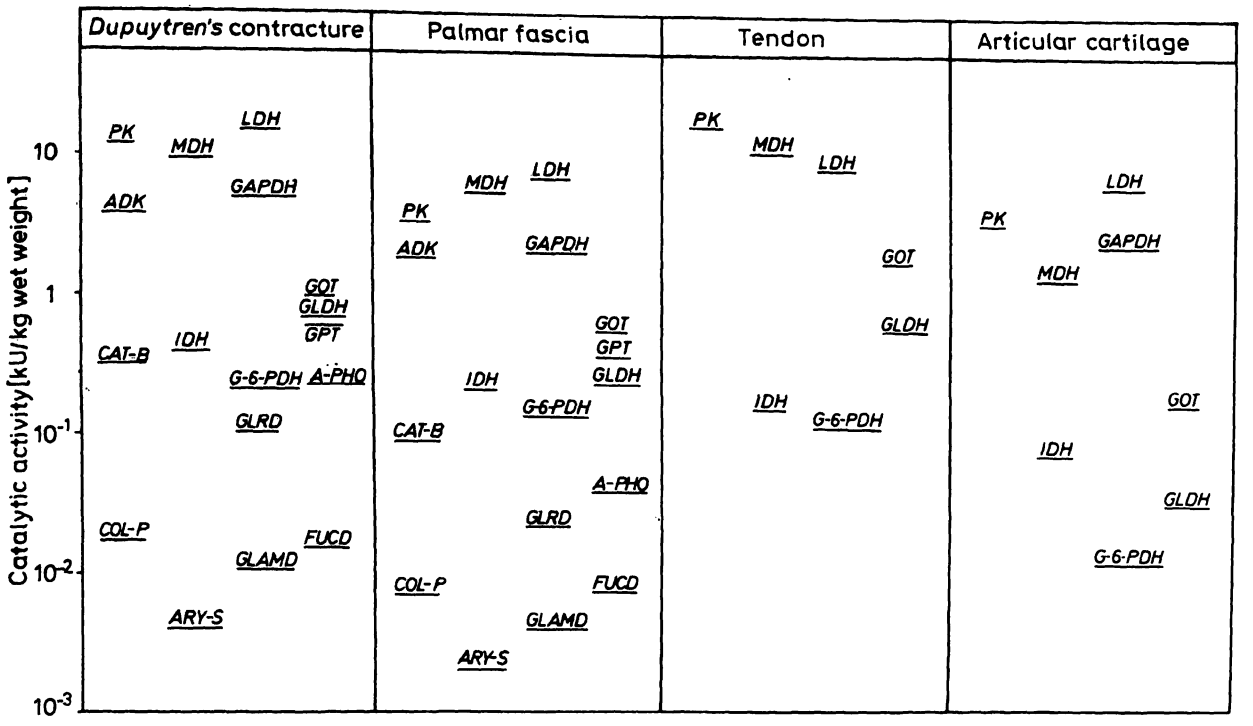

Fig. 2. Enzyme activity distribution patterns from *Dupuytren* contracture and human palmar fascia in comparison to human tendon and articular cartilage (11). Logarithmic scale. Activity in kU/kg wet weight.
Abbreviations: LDH: Lactate dehydrogenase MDH: Malate dehydrogenase GAPDH: Glyceraldehyde-3-phosphate dehydrogenase PK: Pyruvate kinase IDH: Isocitrate dehydrogenase ADK: Adenylate kinase ARS: Arylsulfatase CAT-B: Cathepsin B 1 A-PHO: Acid phosphatase GLAMD: α-N-Acetyl-glucosaminidase COL-P: Collagen peptidase FUCD: α-*L*-Fucosidase GOT: Glutamate-oxalacetate transaminase GPT: Glutamate-pyruvate transaminase GLRD: β-Glucuronidase G-6-PDH: Glucose-6-phosphate dehydrogenase PGK: 3-Phosphoglycerate kinase GLDH: Glutamate dehydrogenase
The line under each abbreviation refers to the enzyme activity (ordinate).

Tab. 2A. Enzyme activities in U/kg wet weight of palmar fascia and of *Dupuytren*'s contracture and subgroups.

	Palmar fascia		Nodular portion *Dupuytren*'s contracture		Thin portion *Dupuytren*'s contracture		Proliferating stage	
	n	$\bar{x}$ $s_{\bar{x}}$ (kU/kg wet weight)	n	$\bar{x}$ $s_{\bar{x}}$ (kU/kg wet weight)	n	$\bar{x}$ $s_{\bar{x}}$ (kU/kg wet weight	n	$\bar{x}$ $s_{\bar{x}}$ (kU/kg wet weight)
Glycerinaldehyde-3-phosphate dehydrogenase	12	1.91 ± 0.15	28	4.90 ± 0.54	14	2.54 ± 0.26	12	4.59 ± 0.88
Adenylate kinase	13	1.80 ± 0.24	30	3.83 ± 0.48	14	1.25 ± 0.23	12	3.06 ± 0.45
Pyruvate kinase	13	3.22 ± 0.39	29	11.6 ± 1.27	15	3.99 ± 0.58	12	10.5 ± 1.5
Isocitrate dehydrogenase	12	0.20 ± 0.041	24	0.38 ± 0.039	10	0.21 ± 0.039	10	0.38 ± 0.059
Malate dehydrogenase	13	5.22 ± 0.56	26	9.23 ± 0.95	9	6.10 ± 0.52	10	7.74 ± 1.05
Lactate dehydrogenase	12	6.49 ± 0.85	31	14.6 ± 1.88	14	4.79 ± 0.95	12	12.8 ± 1.69
Glucose-6-phosphate dehydrogenase	11	0.13 ± 0.032	23	0.20 ± 0.037	11	0.11 ± 0.021	11	0.17 ± 0.041
Glutamate-pyruvate transaminase	12	0.35 ± 0.05	22	0.60 ± 0.12	9	0.45 ± 0.12	10	0.30 ± 0.053
Glutamate-oxalacetate transaminase	11	0.53 ± 0.078	23	0.93 ± 0.13	9	0.64 ± 0.08	9	0.87 ± 0.12
Glutamate dehydrogenase	11	0.22 ± 0.02	22	0.66 ± 0.060	10	0.65 ± 0.1	10	0.72 ± 0.092
		(U/kg wet weight)		(U/kg wet weight)		(U/kg wet weight)		(U/kg weight)
Collagen peptidase	13	7.0 ± 1.94	19	17.1 ± 3.21	5	3.1 ± 1.34	7	14.7 ± 4.54
Cathepsin B 1	4	87.1 ± 22	17	325 ± 37	15	110 ± 19.6	7	381 ± 47.3
Arylsulfatase	9	2.0 ± 1.0	33	3.9 ± 0.52	14	2.1 ± 0.27	12	2.7 ± 0.58
α-*L*-Fucosidase	15	7.2 ± 1.86	32	15.0 ± 2.83	15	5.1 ± 0.77	11	9.7 ± 1.51
β-Glucuronidase	15	21.6 ± 2.84	35	102 ± 9.3	17	54.6 ± 5.58	12	99.0 ± 12.1
α-N-Acetyl-glucosaminidase	14	3.9 ± 1.1	35	10.3 ± 1.01	17	4.1 ± 0.73	12	12.1 ± 2.02
Acid phosphatase	15	37.3 ± 3.9	35	222 ± 37	17	49 ± 9.22	12	186 ± 39.8

Tab. 2B. Levels of significance of enzyme activities per kg wet weight between palmar fascia, *Dupuytren*'s contracture and subgroups.

		Palmar fascia	Nodular portions *Dupuytren*'s contracture	Thin portion *Dupuytren*'s contracture	Proliferating stage	
		p	p	p	p	
Palmar fascia			0.014	0.104	0.156	Collagen peptidase
			0.001	0.461	0.0001	Cathepsin B 1
			0.108	0.981	0.532	Arylsulfatase
			0.02	0.185	0.247	α-*L*-Fucosidase
			0.0001	0.0001	0.0001	β-Glucuronidase
			0.0001	0.866	0,002	α-N-Acetyl-glucosaminidase
			0.0001	0.255	0.003	Acid phosphatase
Nodular portion *Dupuytren*'s contracture	Glyceraldehyde-3-phosphate dehydrogenase	0.000		0.001	0.676	Collagen peptidase
	Adenylate kinase	0.02		0.0001	0.363	Cathepsin B 1
	Pyruvate kinase	0.000		0.01	0.110	Arylsulfatase
	Isocitrate dehydrogenase	0.001		0.003	0.117	α-*L*-Fucosidase
	Malate dehydrogenase	0.001		0.001	0.825	β-Glucuronidase
	Lactate dehydrogenase	0.000		0.0001	0.423	α-N-Acetyl-glucosaminidase
	Glucose-6-phosphate dehydrogenase	0.23		0.0001	0.511	Acid phosphatase
	Glutamate-pyruvate transaminase	0.11				
	Glutamate-oxalacetate transaminase	0.05				
	Glutamate dehydrogenase	0.000				
Thin portion *Dupuytren*'s contracture	Glyceraldehyde-3-phosphate dehydrogenase	0.05	0.000		0.041	Collagen peptidase
	Adenylate kinase	0.11	0.000		0.001	Cathepsin B 1
	Pyruvate kinase	0.12	0.000		0.295	Arylsulfatase
	Isocitrate dehydrogenase	0.95	0.01		0.022	α-*L*-Fucosidase
	Malate dehydrogenase	0.56	0.002		0.004	β-Glucuronidase
	Lactate dehydrogenase	0.36	0.000		0.002	α-N-Acetyl-glucosaminidase
	Glucose-6-phosphate dehydrogenase	0.62	0.007		0.006	Acid phosphatase
	Glutamate-pyruvate transaminase	0.75	0.32			
	Glutamate-oxalacetate transaminase	0.31	0.28			
	Glutamate dehydrogenase	0.001	0.93			
Proliferating	Glyceraldehyde-3-phosphate dehydrogenase	0.002	0.90	0.008		
	Adenylate kinase	0.03	0.43	0.003		
	Pyruvate kinase	0.000	0.66	0.001		
	Isocitrate dehydrogenase	0.04	0.80	0.03		
	Malate dehydrogenase	0.06	0.34	0.1		
	Lactate dehydrogenase	0.002	0.58	0.001		
	Glucose-6-phosphate dehydrogenase	0.49	0.68	0.24		
	Glutamate-pyruvate transaminase	0.69	0.07	0.57		
	Glutamate-oxalacetate transaminase	0.03	0.59	0.13		
	Glutamate dehydrogenase	0.000	0.610	0.62		

phosphate dehydrogenase are much lower and are in the range of the lower limit of analytical accuracy. The enzyme patterns resemble those of other connective tissues such as tendon or cartilage (fig. 2; (29), (30)). The activities of lysosomal enzymes are two to three orders of magnitude lower than the activities of main metabolic pathway enzymes. In palmar fascia and *Dupuytren*'s contracture the activities of cathepsin B 1 and acid phosphatase exceed those of other lysosomal enzymes. The absolute activities calculated on the wet weight basis of the specimens are two to three times higher in *Dupuytren*'s contracture than in palmar fascia. The thin portion of *Dupuytren*'s contracture is significantly different from *Dupuytren*'s contracture and from palmar fascia with respect to the enzyme activities, whereas the proliferating stage does not differ from the total *Dupuytren*'s contracture group but differs from the palmar fascia group. Calculated on the basis of DNA, the absolute activities for both lysosomal and main metabolic pathway enzymes are somewhat higher in palmar fascia than in *Dupuytren*'s contracture (tab. 3). Enzyme activities based on the glyceraldehyde-3-phosphate dehydrogenase activity show no statistically significant differences in specimens from *Dupuytren*'s contracture or palmar fascia (tab. 4, fig. 3).

The absolute activities as well as the enzyme activity distribution patterns of fibroblasts from palmar fascia and *Dupuytren*'s contracture are identical. In particular, the activities of lysosomal enzymes do not differ significantly between both strains of cultured fibroblasts. The activity distribution patterns (based on the activity of glyceraldehyde-3-phosphate dehydrogenase) of main metabolic pathway enzymes from fibroblasts and the respective tissue specimens from palmar fascia and *Dupuytren*'s contracture (fig. 3) differ only slightly. While adenylate-kinase and pyruvate-kinase are lower, lactate dehydrogenase and glucose-6-phosphate dehydrogenase show a higher activity in fibroblasts than the corresponding tissue specimens (statistical significance see table 3). Due to the small number of samples of palmar fascia cell strains tested, the significance of these differences are less than those for *Dupuytren*'s contracture tissue specimens and *Dupuytren*'s contracture cell cultures. However, lysosomal enzyme activity patterns are different for tissue specimens and cultured fibroblasts. In general, the activities of these enzymes are higher in relation to the main metabolic pathway enzymes in tissue specimens than in the respective cultured fibroblasts. This is especially true for β-glucuronidase and α-N-acetyl-glucosaminidase (fig. 3).

A great difference in overall activities could be shown by comparing tissue enzymes with cultured fibroblast enzymes on the basis of the DNA content. Enzyme activities per g DNA are 10 to 50 times higher in fibro-

Tab. 3. Enzyme activities in cultured fibroblasts from *Dupuytren*'s contracture and palmar fascia. U/U glyceraldehyde-3-phosphate dehydrogenase. Significance level of differences between both fibroblast groups (A); between fibroblasts from *Dupuytren*'s contracture and *Dupuytren*'s contracture tissue (B); and fibroblasts from palmar fascia and palmar fascia tissue (C) (see table 2A).

	Fibroblasts Palmar fascia		Fibroblasts *Dupuytren*'s contracture		Significance level		
	n	$\bar{x} \pm s_{\bar{x}}$ (U/U Glyceraldehyde-3-phosphate dehydrogenase)	n	$\bar{x} \pm s_{\bar{x}}$ (U/U Glyceraldehyde-3-phosphate dehydrogenase)	A	B	C
Adenylate kinase	5	0.29 ± 0.05	27	0.36 ± 0.03	n.s	< 0.0001	< 0.001
Pyruvate kinase	6	0.99 ± 0.33	31	0.98 ± 0.12	n.s	< 0.0001	< 0.02
3-Phosphoglycerate kinase	4	1.49 ± 0.09	27	1.84 ± 0.14	n.s	–	–
Lactate dehydrogenase	5	4.29 ± 0.29	29	4.05 ± 0.2	n.s	< 0.001	< 0.2
Glucose-6-phosphate dehydrogenase	5	0.16 ± 0.03	22	0.13 ± 0.02	n.s	< 0.0001	< 0.02
		(mU/U Glyceraldehyde-3-phosphate dehydrogenase)		(mU/U Glyceraldehyde-3-phosphate dehydrogenase			
Collagen peptidase	5	3.23 ± 0.38	27	3.63 ± 0.22	n.s	n.s.	n.s.
Arylsulfatase	4	1.36 ± 0.85	24	0.85 ± 0.05	0.02	< 0.02	n.s.
β-Glucuronidase	5	3.71 ± 1.66	27	4.09 ± 0.31	n.s	< 0.0001	< 0.01
α-N-Acetyl-glucosaminidase	5	0.24 ± 0.05	25	0.26 ± 0.04	n.s	< 0.0001	< 0.002
Acid phosphatase	5	15.2 ± 2.72	25	13.6 ± 1.3	n.s	< 0.01	< 0.2

Tab. 4. Comparison of enzyme activities calculated on the DNA basis in specimens from *Dupuytren*'s contracture and normal palmar fascia.

	Palmar fascia			Significance level	*Dupuytren*'s contracture		
	n	$\bar{x}$	$s_{\bar{x}}$	p	n	$\bar{x}$	$s_{\bar{x}}$
		(kU/g DNA)				(kU/g DNA)	
Glyceraldehyde-3-phosphate dehydrogenase	13	14.8	± 3.2	0.02	25	8.52	± 0.8
Adenylate kinase	13	13.2	± 2.1	0.1	27	6.25	± 0.6
Pyruvate kinase	13	25.3	± 5.4	0.2	23	20.5	± 1.7
Isocitrate dehydrogenase	13	1.61	± 0.3	0.002	21	0.61	± 0.08
Malate dehydrogenase	13	42.3	± 9.3	0.001	18	17.9	± 1.96
Lactate dehydrogenase	13	46.9	± 9.7	0.01	28	23.7	± 2.4
Glucose-6-phosphate dehydrogenase	13	1.04	± 0.3	0.05	19	0.39	± 0.08
Glutamate-pyruvate transaminase	13	2.36	± 0.4	0.01	22	1.08	± 0.25
Glutamate-oxalacetate transaminase	13	4.10	± 0.9	0.01	22	1.58	± 0.21
Glutamate dehydrogenase	13	1.72	± 0.4	0.1	18	1.11	± 0.14
		(U/g DNA)				(U/g DNA)	
Collagen peptidase	11	16.8	± 5.4	0.71	18	19.4	± 4.34
Cathepsin B 1	7	374	± 88	0.45	15	300	± 51
Arylsulfatase	11	5.3	± 1.9	0.15	28	3.3	± 0.49
α-*L*-Fucosidase	17	44	± 24	0.09	26	9.7	± 1.57
β-Glucuronidase	15	117	± 33	0.37	29	92	± 10.7
α-N-Acetyl-glucosaminidase	15	23.4	± 8.3	0.04	29	10.1	± 1.5
Acid phosphatase	16	148	± 37	0.35	29	223	± 55

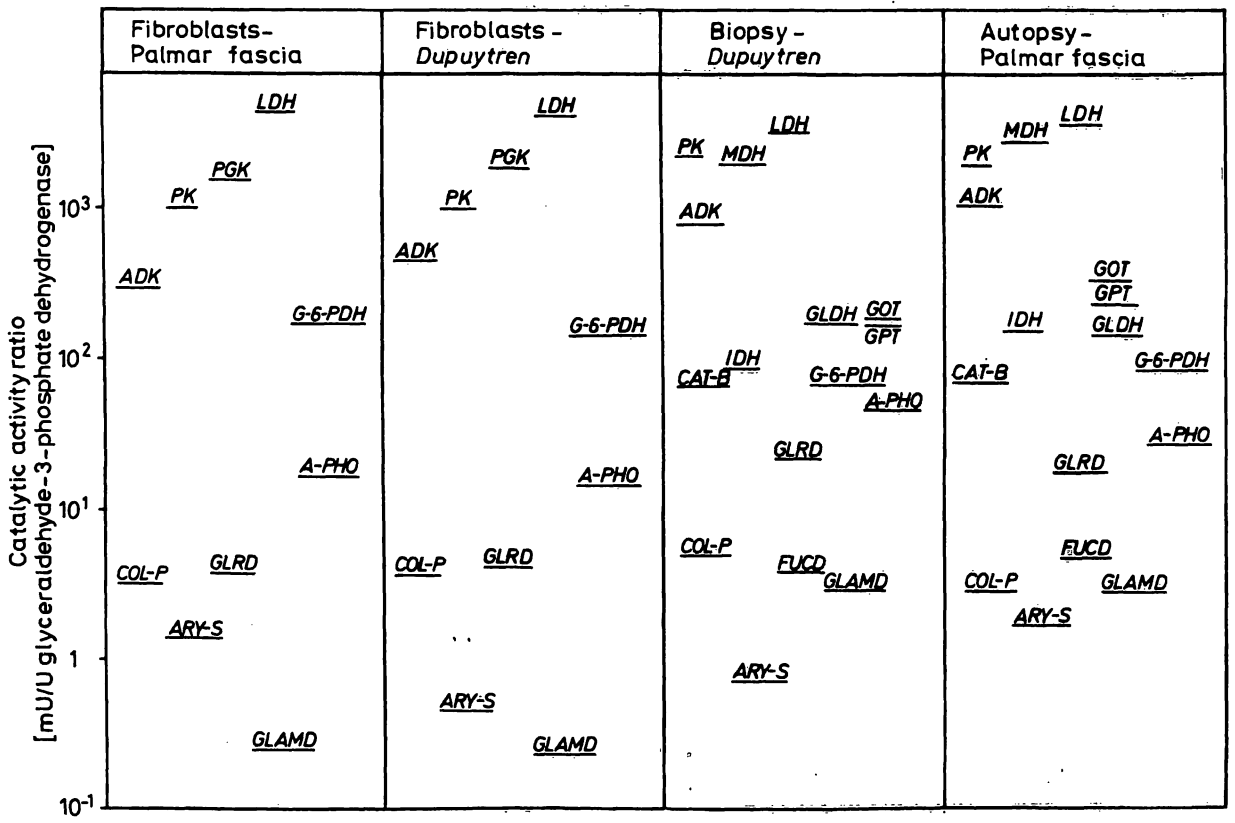

Fig. 3. Comparison of enzyme activity distribution patterns from cultured fibroblasts (*Dupuytren*'s contracture, palmar fascia) with the respective patterns of tissue specimens on the basis of glyceraldehyde-3-phosphate dehydrogenase activity, Logarithmic scale. Explanations see fig. 2.

blasts depending on the duration of the growth period and/or the density of fibroblasts in the cell layer (fig. 4, 5). This applies to *Dupuytren*'s contracture and *Dupuytrens*'s contracture fibroblasts, and to palmar fascia and palmar fascia fibroblasts. During the course of cell growth, enzyme activities and protein content of the cells decrease while the DNA content remains almost unchanged (fig. 5). Enzyme activities closely correlate with the protein content of the cells (for lactate dehydrogenase/protein r = 0.91 for n = 24).

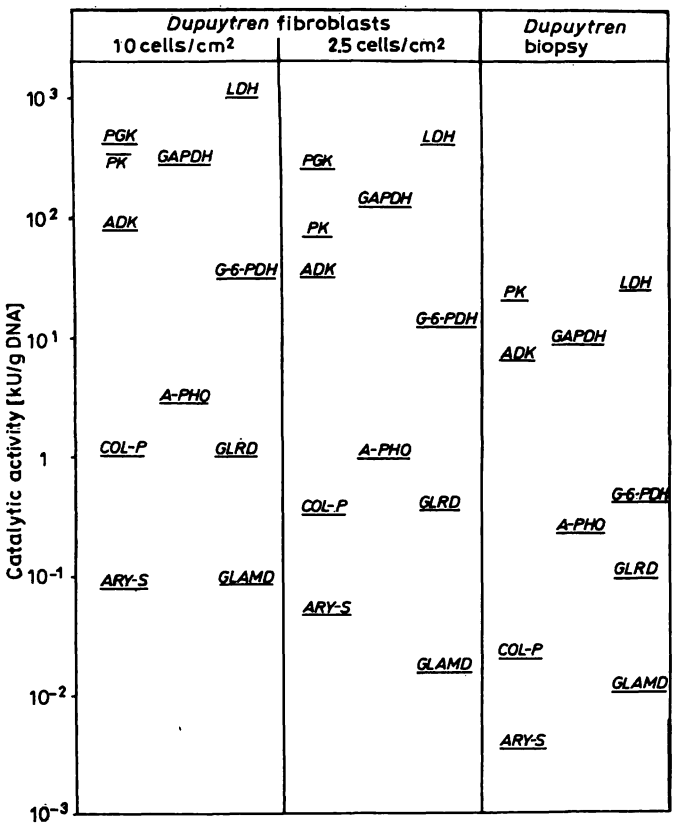

Fig. 4. Enzyme activity distribution patterns on the basis of the DNA content in cultured fibroblasts of different cell density in comparison to the respective tissue specimens. Logarithmic scale. Explanations see fig. 2.

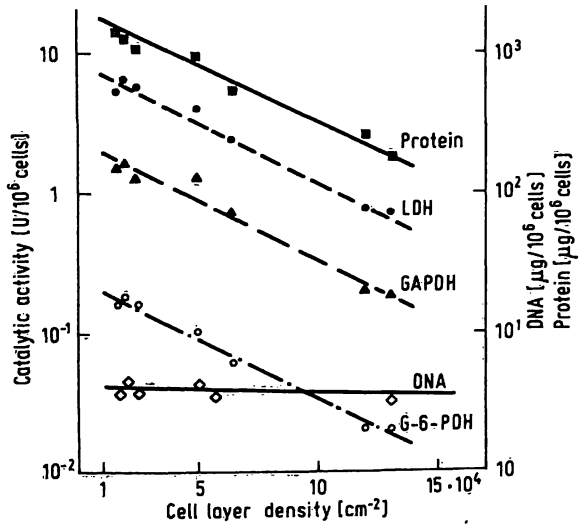

Fig. 5. Enzyme activities, extractable protein and DNA contents of cultured fibroblasts in dependence of the cell layer density. Activity in U/10^6 cells, protein and DNA in µg/10^6 cells. Logarithmic scale. Abbreviations see fig. 2.

Discussion

Since the tissue samples were homogeneous and the blood impurities negligible (0.01%), the possibility of falsification of the enzyme patterns by enzymes from other sources was excluded. However, due to the difficulties of equally disrupting strong connective tissue portions, the scatter of the results within the individual groups is relatively high. A mean of 90% of the total enzyme activities was extracted by the method applied in this study. On the other hand, fibroblast extraction leads to more precise results with smaller deviation from the mean. The latter can be achieved only when fibroblasts of the same cell density in culture are taken for extraction. The methodological scatter of the results partly explains the poor significance of differences between the groups for those enzymes with low activities in the tissues. For interpretation of the data on enzyme activities, the metabolic capacity of the respective tissue as well as that of the individual cell must be taken into consideration. The basis for a comparative interpretation should be first the wet weight, then the DNA content or the cell number. Extracted protein, however, did not prove to be a suitable reference in the tissues under investigation. The missing correlation between values for extracted protein and DNA content (fig. 1) shows that, in addition to the metabolically active enzyme proteins, other proteins such as neutral salt-soluble collagen are extracted under the experimental conditions applied in this study. Due to the constant correlation of enzyme activities in different tissues, extractable enzyme proteins offer a further possibility of a reference system (31). For instance, glyceraldehyde-3-phosphate dehydrogenase as a key enzyme of glycolysis (32) could be used as reference for the comparison and interpretation of enzyme patterns of the specimen groups under discussion.

The data on the enzyme activities in palmar fascia and *Dupuytren*'s contracture allow the conclusion that the higher metabolic activity of *Dupuytren*'s contracture does not depend on changes in the enzymatic activities within the individual cell, but rather on the larger number of cells present in the diseased portions of the tissue, as seen in the morphological examination of the *Dupuytren*'s contracture samples (3). These findings are in agreement with the higher DNA content of the *Dupuytren*'s contracture samples compared with palmar fascia specimens. However, the enzyme activities based on the DNA content are lower in *Dupuytren*'s contracture than in palmar fascia. These differences might be explained by a higher DNA content per cell in proliferating cells of *Dupuytren*'s contracture compared to the non-proliferating cells in palmar fascia. On the other hand, preliminary experiments on main metabolic pathway enzyme activities in growing fibroblasts (fig. 5) showed a decrease in enzyme activity and protein content per cell of cultured fibroblasts with increasing age and density of cells. If cell density is the regulating factor

for the activity of cell enzymes, the difference in enzyme activity per DNA (or cell) could be attributed to the isolated localisation of cells in palmar fascia in contrast to the proliferating cell clusters in *Dupuytren*'s contracture (see below).

Although histologically *Dupuytren*'s contracture exhibits tissue breakdown alongside tissue regeneration, the activities of lysosomal peptidases (cathepsin B 1, collagen peptidase) involved in collagen breakdown (33, 34, 25), the carbohydrases and the acid phosphatase do not significantly differ between palmar fascia and *Dupuytren*'s contracture on the tissue DNA basis. This means that the elevated metabolic turnover of extracellular compounds is provided by an increased cell number in *Dupuytren*'s contracture.

The data of *Hoopes* et al. (12) differ considerably from the results of this study. Based on the DNA content, the authors reported an increase in enzyme activities in specimens of *Dupuytren*'s contracture which varied between 1 and 10 times from enzyme to enzyme. However, the methods applied for enzyme activity determinations were different. *Hoopes* et al. incubated tissue slices instead of testing tissue extracts which means that enzymes or substrates have to penetrate extracellular matrix and fibres prior to enzymatic action on the substrates. This extracellular compartment is assumed to be a stronger barrier for both enzymes and substrates in the case of the intact palmar fascia than in the pathological structure of *Dupuytren*'s contracture. In addition, the penetration rates of the different substrates or enzyme proteins may vary, thus causing different elevation rates of individual enzymes as reported by the authors. Calculating the activities determined by *Hoopes* et al. on the basis of glyceraldehyde-3-phosphate dehydrogenase, the differences in enzyme activities are in the range of 1–2 fold, depending on the activity of glyceraldehyde-3-phosphate dehydrogenase given by the assay method. Moreover, tissue slices contain more or less intact cells, subcellular microstructures, and substrates in various concentrations, providing metabolic reaction sequences which may interfere with the specific enzyme reactions to be determined. These methodological differences apply also to the data published by *Hoopes* et al. on enzyme activities in hyperytrophic scars and keloids (36). The difficulties involved in the techniques used by the authors are evident by the fact that, in normal dermis, no measurable glyceraldehyde-3-phosphate dehydrogenase activity was detected; this seems very unlikely in view of the high activities of other enzymes of the *Embden-Meyerhof* pathway in the examined specimens. Although the data of *Hoopes* et al. are not comparable to the results given in this paper, they do demonstrate a considerable metabolic activity of palmar fascia and *Dupuytren*.

Studies on the synthesis of collagen and acid glycosaminoglycans in cultured fibroblasts have shown an enhanced [^{3}H]hydroxyproline and [^{35}S]sulfate incorporation in cells cultured from *Dupuytren*'s contracture biopsies compared to palmar fascia fibroblasts (26). Corresponding differences in the determined enzyme activities are missing in the respective fibroblasts (fig. 3). However, there are some distinct differences between enzyme activities in fibroblasts and the respective tissue specimens from which fibroblasts were derived (fig. 3, tab. 3), with respect to the overall activity and the relationship of the activities of lysosomal enzymes to those of main metabolic pathway enzymes. These differences very probably have a methodological basis. In the case of the tissue specimens, the extraction procedure includes the extracellular compartment, and this may contain varying relative quantities of individual secreted lysosomal enzyme proteins. However, the fibroblasts were isolated from the surrounding medium prior to the extraction. In this way the extracellular portion of the lysosomal enzyme activities was removed before activity determinations. Enzyme activity determinations in isolated cells offer the opportunity to distinguish between intracellular activity of lysosomal enzymes and the activity of the secreted enzyme proteins. When setting up cell culture experiments along with enzyme activity assays in tissue specimens, it should be possible to answer the question whether enzyme activities derive from the specific cells of the respective tissue or originate from other sources such as granulocytes or macrophages. No final explanation can be offered as to the differences in enzyme activity per g DNA between tissue and the respective cultured fibroblasts (fig. 4). Experimental errors in DNA determination can be excluded since the respective data from tissue and cultured fibroblasts concur with those of the literature (12, 37). The data on the dependence of enzyme activity upon cell density in cultured fibroblasts suggest a mechanism by which the enzyme activity is regulated by extracellular factors, such as cell to cell interaction or the state of extracellular matrix. Future studies must be performed to reveal more detailed information on this matter and prove the possibility of establishing similar data in in vivo systems of proliferating cells. For the present, it must be emphasized that work on the metabolism of fibroblasts, using cultured cells, should take into consideration the comparability of the state of cell growth and the density of the cells under investigation.

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